TranSignal™ TF Protein Array

Cat. # MA3501 - MA3508 Product User Manual Released 03/22/05 Revised 08/11/05



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1. INTRODUCTION

The human genome comprises an estimated 30,000 genes, 2,000 of which encode proteins involved in the control of gene expression. These proteins, known as transcription factors (TFs), are responsible for the who, what, when, where, and how of gene expression: which genes are expressed in what cell types, under what environmental conditions, and at what stages of development. The activity of TFs is highly regulated by various mechanisms, including protein induction, modification, translocation, degradation, and inhibition (1).

Before activated TFs can impose regulation on the basal transcriptional machinery, they either bind to their corresponding specific *cis*-elements, or interact with other TF proteins. This interaction usually requires cofactors to bridge the TF proteins and the components of the basal transcriptional machinery. The interactions between TFs, cofactors, and basal transcriptional machinery create a complex, multidimensional network (2).

Identifying and characterizing the interaction network in the context of different cellular environments will allow us to understand the network structure, as well as the cellular signal-induced changes in this structure. Tools for studying protein-protein interactions—especially interactions involving TF proteins—are essential for understanding gene expression and regulation.

Novel method for investigating protein-protein interactions

Traditionally, protein-protein interactions have been studied by pull-down assay, coimunoprecipitation, super-gel shift, and the yeast two-hybrid system. But because these methods are notoriously time-consuming and inefficient, they are not conducive to mapping the intricate network of protein-protein interaction. That's where the TranSignal TF Protein Array comes in. This method enables you to determine how a particular protein interacts with multiple other proteins—in a single detection experiment.

Figure 1 illustrates how this simple procedure works. The array membrane is spotted with transcription factor proteins, which are expressed from full length TF cDNAs, with an N-terminal His Tag. A protein of interest is

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TranSignal™ TF Protein Array

used as bait to search for interactions with the immobilized proteins. Interactions can be assessed either by using an antibody to the protein of interest, or with antibody to protein tags such as HA, GST or biotin. The signal is visualized via chemiluminescent detection.

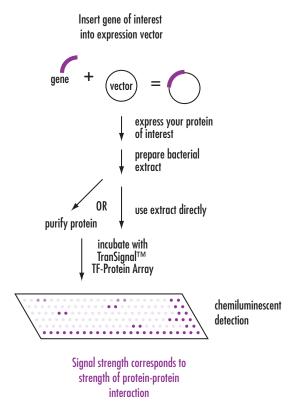


Figure 1: Flow chart of the TranSignal $^{\text{TM}}$ TF Protein Array protein interaction

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TranSignal™ TF Protein Array

Novel method to characterize gene promoter regions

The TranSignal The Protein Array provides a method by which you can very quickly survey direct interactions between TF proteins and their complimentory cis-binding element. This means that by simply producing a biotinylated version of your promoter region of interest you can asses which transcription factors arrayed on the membrane have the potential to bind to this region. Once these have been identified it starts to give clues about which transcription factors, and their associated signaling pathways, might be regulating the expression of your gene of interest.

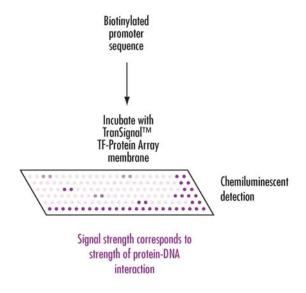


Figure 2: Flow chart of the TranSignal TF Protein Array DNA Promoter interaction

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2. CHOOSING AN ANTIBODY DETECTION METHOD

IMPORTANT: Read this section before beginning your experiment!

To detect interactions between your protein of interest and the TF proteins immobilized on the membrane, you will need to use either an antibody against your protein of interest or a tag antibody, such as HA, biotin or GST. As a negative control, incubate the TranSignal TF Protein Array membrane with antibody only (see Appendix B).

2.1 Using an antibody against your protein of interest

If you already have access to your purified protein of interest or bacterial lysate containing the protein of interest, you can get started right away. Simply incubate the array membrane with your purified protein, then detect using the antibody.

2.2 Using a protein tag/antibody combination

If you don't have an antibody against your protein of interest, you will need to use protein with a specific tag, such as HA or GST or biotinylate your protein. **Do not use His tagged proteins for hybridization with the membranes as the proteins on the array are His tagged.** You can then detect hybridized proteins using an antibody against the tag.

2.3 Using a protein tag/antibody combination

We provide a suitable streptavidin - HRP conjugate for use with this kit

3. MATERIALS PROVIDED

For Catalogue items MA3501, MA3502, MA3503 and MA3504 Protein - Protein interactions.

STORAGE CONDITONS: Upon receipt, store array membranes, and all reagents at 4° C. Keep at 4° C until use.

- TranSignal TF Protein Array (2 each)
- 1X Blocking Buffer I (30 ml)
- 1X Blocking Buffer II (30 ml)
- 20X Wash Buffer (20 ml)-dilute to 1X with dH₂0

- Detection Buffer A (600 µl)
- Detection Buffer B (600 µl)

For Catalogue items MA3505, MA3506, MA3507 and MA3508 DNA - Protein interactions.

STORAGE CONDITONS: Upon receipt, remove the 5X Blocking Buffer and store at -20°C. Store array membranes, and all other reagents at 4°C.

- TranSignal TF Protein Array (2 each)
- 4 well plate (1 each)
- 5X Binding Buffer (5 ml)
- 20X Wash Buffer (20 ml)-dilute to 1X with dH,0
- Streptavidin HRP (20 µl)
- Detection Buffer A (600 µl)
- Detection Buffer B (600 µl)

Sufficient quantities of each buffer are provided for two assays.

4. ADDITIONAL MATERIALS REQUIRED

- 4.1 Reagents and Solutions
 - Appropriate primary detection antibody
 - Anti-rabbit, mouse or goat HRP conjugate
- 4.2 Materials and Equipment
 - Microcentrifuge
 - Orbital shaker
 - Plastic Sheets (e.g., overhead transparencies)
 - Hyperfilm™ ECL (Amersham, Cat.# RPN1674K) or equivalent OR
 - Chemiluminescence imaging system (e.g., FluorChem™ from Alpha Innotech Corp.)

5. INCUBATION OF PROTEIN WITH THE ARRAY MEMBRANE

Use this protocol with catalogue items MA3501, MA3502, MA3503 and MA3504.

This section describes incubating bacterial extract containing your protein of interest or purified protein with the array membrane. Note that the array membranes are notched at the top right-hand corner for orientation purposes.

Note: Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.

- 5.1 Place each membrane into a container containing 4 ml of 1X Blocking Buffer I.
- 5.2 Place the tray on a shaker and incubate for 2 hr at room temperature.
- 5.3 Dilute the bacterial extract, containing overexpressed protein to a final concentration of 60 μ g/ml in 4 ml of 1X Blocking Buffer I. If you have purified protein, dilute 3 -5 μ g purified protein in 4 ml of 1X Blocking Buffer I.
- 5.5 Incubate the membrane with the diluted bacterial extract or purified protein at room temperature for 2 hr with gentle shaking.
- 5.6 Wash the membrane twice with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.
- 5.7 Incubate the membrane with 4 ml of 1X Blocking Buffer II containing primary antibody:
 - **Note:** Use an amount of the antibody suitable for western blot detection of the protein or protein tag, specified by the manufacturer.
- 5.8 Incubate the membrane with the primary antibody solution for 2 hours at room temperature,
- 5.9 Wash the membrane twice with 4ml 1X Wash Buffer for 5-min (each wash).
- 5.10 Incubate the membrane with the appropriate secondary antibody diluted 1:5,000–1:15,000 in 4ml 1X Blocking Buffer II for 1 hr at room temperature.
- 5.11 Wash the membrane four times with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.

6. DETECTION

Important: Do not let the membrane dry out during detection.

- 6.1 Prepare 500μl of detection solution by combining 250μl Detection Buffer A and 250 μl of Detection Buffer B per membrane.
- 6.2 Using forceps to hold the notched corner, carefully remove each membrane from its tray. Drain the excess Wash Buffer from the membrane by touching the edge against tissue. Place membrane protein-side-up on a clean plastic sheet protector or overhead transparency, by orienting the notch to the top, right-hand corner.
- 6.3 Pipet the mixed Detection Buffers onto the membrane. Overlay the membrane with a second plastic sheet and ensure that the buffer mixture is evenly distributed over the membrane without air bubbles.
- 6.4 Incubate for 5 min at room temperature.
- 6.5 Remove excess substrate by gently applying pressure to the top sheet. Using a paper towel, remove excess detection solution remaining on the surface of the sheets.
- 6.6 Expose the membranes using either Hyperfilm[™] ECL or a chemiluminescence imaging system, such as the FluorChem[™] imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposures of varying lengths of time (e.g., 30 sec−5 min).

7. INCUBATION OF DNA WITH THE ARRAY **MEMBRANE**

Follow this protocol for use with catalogue items, MA3505, MA3506, MA3507 and MA3508

This section describes incubating a biotinylated oligo of interest with the array membrane. We recommend that you use a 5'biotinylated Oligo for

Note: Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.

- Place each membrane into a container containing 4 ml of 1X Binding Buffer.
- 7.2 Place the tray on a shaker and incubate for 2 hr at room temperature.
- 7.3 Dilute 50 200 ng of biotinylated DNA probe in 4 ml of 1X Binding Buffer. For the competition assay cold probes should be in at least 5 X excess of the biotinylated probe (0.25-1 µg)
- 7.5 Incubate the membrane with the diluted DNA probe at room temperature for 30 min with gentle shaking.
- 7.6 Wash the membrane two times with 4 ml of 1X Wash Buffer for 3 min (each wash) at room temperature.
- 7.7 Dilute the Streptavidin HRP (1:1000 fold) in 1X Wash Buffer and incubate the membraneg with the diluted Streptavidin - HRP for 30 min at roomtemperature.
- 7.8 Wash three times with 4 ml of 1X Wash Buffer for 5 min (each wash) at room temperature.
- 7.9 Continue with step 6.1 for detection.

8. TROUBLESHOOTING GUIDE

Problem	Cause	Recommendation		
Weak or no signal	Not enough protein.	Check protein concentration by running the sample on SDS-PAGE.		
		Check construct by DNA sequencing. Make sure the DNA insert is in the right fran and that the protein expresses properly.		
	Tag is partially hidden.	Protein binding may be hindered by a partially hidder tag. Try using a higher concentration (5-10X of the bacterial lysate) or longer binding time.		
High background	Concentration of bacterial lysate is too high.	Further dilute bacterial lysate or use purified protein.		
	Antibody concentration is too high.	Further dilute the antibody.		
	too mgn.	Dilute the Detection Buffer.		
Uneven background	Membrane dried out during incubation.	Keep the membrane fully submerged in solution during all incubation steps.		
	Volume of blocking solution, bacterial lysate or antibody is too low.	Increase the volume to make sure that the membrane is full submerged during incubation		
	Volume of detection buffer is too low.	Increase the volume to make sure that the membrane surfactis fully covered.		
	Air bubbles on membrane surface during detection.	Remove air bubbles from membrane surface.		

REFERENCES

- 1. Sawyer TK (2001) Decifering therapeutic targets. BioTechniques 30: 1086-1090.
- 2. Chan, H M., and La Thangue, N. B. (2001) p300/CBP protiens: HAT for trancriptional bridges and scaffolds. J Cell Science 114: 2363-2373.
- 3. Chunhong Yan, Heng Wang, and Douglas D. Boyd (2002) ATF3 Represses 72-kDa Type IV Collagenase (MMP-2) Expression by Antagonizing p53dependent trans-Activation of the Collagenase Promoter. J Bio Chem 277: 10804-10812.

APPENDIX A: Schematic diagram of the TranSignal TF Protein Array Version I

	~	_				ш.	
24	Sod	Sod	Sod	Sod	Sod	Sod	24
23	pos	Sod	pos	pos	pos	pos	23
77	C/EBPα	DRI	ESI	GTE28		DOS .	22
71	(/EBPalc/	DRI	ETSI	GTF28		pos	21
70	8162	HIMO	ERRy	68		pos	20
19	8162	LJIWO	ERRY	6R		Sod	19
<u></u>	1378	þXTO	gh1	GMEBI		bos	22
	1378	þXTO	glæ	[8]W9		sod	11
91	ATF4	81100	ERα	BONF		sod	92
]5	ATF4	E1100	Rα	GONF		Sod	15
14	ATF3	Ж	EGR4	GANI		pos	7
33	ATF3	Ж	6684	GATA1		pos	13
13	MF2	CRSP9	EGR2	FOS12		pos	13
=	AF2	CRSP)	EGR2	FOSL2		pos	=
0	MF1	CREM	EGRI	FOSLI		p0S	2
6	MF1	CREM	EGR1	FOSL1		pos	6
∞	ASH21.	CKB812	EZF6	FOSB	IMD]	pos	
7 8	ASH21.	CRBL2	E2F6	FOSB	HANDI	pos	7
9	IDSV	CREBI	5123	FOS	GTF3C5	pos	9
2	IDSV	CREBI	5173	FOS	GTF3C5	sod	5
3 4 5 6	μZα	OXZ	E2F4	F2RL	GFF21	pos	4
	AP2α	CXX	F2F4	F2RL	GFF21	pos	~
1 2	SW	3480	EZE3	ETSZ	I2 GTF2H2	pos	2
-	AES	889	E2F3	ETSZ	GFE 2H2	Sod	_
	-	-	_				

Schematic diagram of the TranSignal TF Protein Array. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

APPENDIX B: Schematic diagram of the TranSignal TF Protein Array Version II

	⋖	8	J	0	ш	-	
24	Sod	Sod	Sod	Sod	Sod	Sod	24
23	sod	pos	Sod	pos	pos	pos	23
11	RFI	MADH3	NFE2	PAUM		Sod	77
71	IKI	MADH3	ZEJN	AUA		pos	71
70	103	MADHI	[<u>@</u>	p23		p05	70
119	101	MOHI	M8]	p33		p05	10
<u>@</u>	101	[H]	l/S/I	NR5A2		pos	∞
13 14 15 16 17 18		[M]	l/S/I	NR542		pos	
91	HOXCII	LEXZ	WEZD	NR2E1		pos	91
15	HOXCII	LHX2	MEF2D	NR2E1		pos	15
14	TOXOH ETBXOH ETBXOH	1081	MEF2C MEF2D	NR113		p0S	7
13	ELØXOH	1081	MEF2B MEF2C	NRTIB		pos	33
11	HOXAS	KEF	MEF28	NRTIZ		pos	11
=	HNF46 HOXAS HOXAS	KLF7	MEF2A MEF2B	NRTIZ		pos	=
9	HNF46	KLF12	MEF2A	NR1H2		pos	=
6	HNF46	KLF12	MEF2A	NFYB NR1H2		p0S	6-
~	λЖ	JUNB	MECP2	NFY8		pos	~
1	Ш	9NOT	WECP2	NFYB		sod	_
9	HOXAS	MI	XW	NFxBp65	PBX1	Sod	9
2	HOXA5 HOXA5	JUN	XW	Wikdp50 Wikdp65 Wikdp65	PBX1	pos	4 5 6 7
3 4 5 6 7 8	LOWGH	SII	MAFK	NFx8p50	PAX9	pos	4
~	HDWC1	IISI	MARK	NFx8p50	PAX9	Sod	~
2	ZONH	ISGF36	MADHA	NFIL3	PAX6	pos	2
_	HAND2	SGF3G	MADHA	NFIL3	PAX6	pos	_

Schematic diagram of the TranSignal TF Protein Array. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

APPENDIX C: Schematic diagram of the TranSignal TF Protein Array Version III

	₩	-	J				
74	Sod	pos	Sod	Sod	Sod	Sod	24
73	bos	DG.	bos	bos	bos	рог	23
\mathbb{Z}	PURA	SIXZ	SURB7	v-Myc		DOC	11
11	PURA	SIX2	SURB7	N/V		pos	21
20	79111	黑	9MIS	TRIP13		pos	20
96	79IIJ	₩	9MIS	TRIP13		DOC	66
22	PTTGI	SOME	SINA	TROP		Sod	22
	PTT61	SCML1 SCML1	NINS.	IRO		Sod.	11
91	PTGER2	K/Bb	STAT3	2		SOL.	91
15	PTGER2	WBb WBb	STATS	2		Sod	15
7	PS/I/C5	988	LIMIS	=		Sod	7
13	PSINCS	988	LIMIS	M		Sod	33
13	PPARYZ PPARYZ PSINCS	RWA	SW.	III		Sod	113
=	PPRYZ	RXRA	ANS.			bd	=
0.	PMRyl	RWIB	NS.	置		Sol	9
6	PPARyi	RXR1B	84	選		Sod	6
~	PPARIG	RFXANK	SF3	EE		<u>SS</u>	~
_	PMRB	RFXANK	SPS	噩		Sol	_
9	PMRa	REVERB	lß	FD		Sod	9
5	PPARC	REVERB	SP1	IF2D		<u>SS</u>	2
4	POUZAFI	Æ	SMARCEI SAMRCEI	TCF712		Sod	4
~	POUZAFI	팶	SIMARCET	TCF712		Sod	~
1	PFDN4	PXR2	LOWNIS	TŒA1	Ш	Sod	1
-	PFDN4	PXR2	SMARCBI	TCEAI	Ш	Sod	_
	-	9	$\overline{}$				

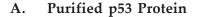
Schematic diagram of the TranSignal TF Protein Array. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row F) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

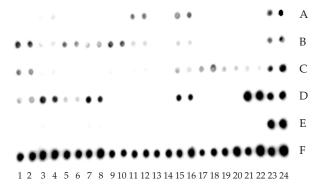
APPENDIX D: Schematic diagram of the TranSignal TF Protein Array Version IV

	-	-	J	_	ш.	
74	Sod	Sod	Sol	Sod	Sod	74
23	sod	pos	Sod	pos	Sod	23
11	EGR1		PMRB		Sel	11
21	1893		PMRB		<u>SOL</u>	11
07	6214		PPARCC		50d	07
19	ЫÜ	III	Pilka		Sod	19
82	E3E3	HNF46	X	W	<u>sa</u>	82
11	E3E3	HNF46	X	M	Sod	11
16		85	%W	vHyc vHyc	<u>S</u>	91
15	[88)	99	PW/8	NIII)C	Sod	15
14	7/00	GATA 1	NFx8p65 NFx8p65	SIMI	boc	7
13	ZXO	GATA1	NFx8p65		Sod	13
13	WB/)	E088	NFA8950	SP4	Sod	11
=	V.BPn (V.BPn	FOSB	NFx8p60	SP4	<u>sa</u>	=
9	ATA	SS	NE2	SH.	Sod	2
6	AF4	FIS	ME2	S	SE.	6
~	ATF3	ETSZ	WEF28	RXR:A	Sod	~
1	ATF3	EISZ	MEF28	RXR.A	<u>sa</u>	1
9	AF2	ESI	MEF2A	PXR:2	<u>s</u>	9
2	AF2	ISI	MEF2A	PXR:2	Sod	5
4	W.	EGR4	¥	PMRy2	Sod	4
~	MFI.	EGR4	M	1 PPARY2 PPARY2 1	<u>sa</u>	~
7	AP2a	EGR2	S	PMRyl	Sod	7
-	AP2a	EGR2	S	PMRyi	Sod	_
	4	8	J	0	ш	

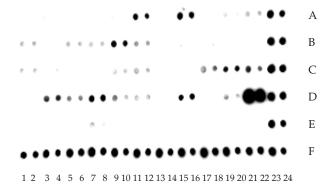
Schematic diagram of the TranSignal TF Protein Array. The proteins on the array are spotted in duplicate. HRP marker has been spotted along the bottom (row E) and in duplicate along the right side (column 23, 24) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.

APPENDIX E: Typical Results of the TranSignal TF Protein Array when screening protein - protein interactions





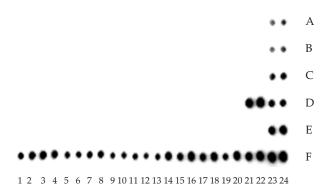
B. Bacterial Lysate containing p53



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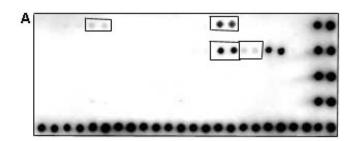
For Technical Support, call 1.877.726.6642 (PANOMIC) or visit our Web site at www.panomics.com.

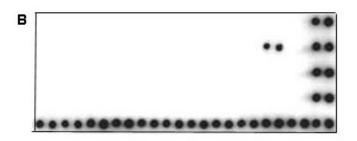
Negative Control



Typical results obtained with the TranSignal TF Protein Array. p53-expressing bacterial $lysate (A), p53 \ purified \ protein (B), and \ buffer \ only (C) \ were \ incubated \ with \ the \ TranSignal \ TF$ Protein Array membrane for 2 hours and detected by p53 monoclonal antibody (Upstate Biotechnology) and anti-mouse Ig HRP conjugate (Amersham). Images were acquired using FluorChemTM imager (Alpha Innotech). As reported in the literature, ATF3 (position A11, A12) interacts with p53 (3).

APPENDIX F: Typical Results of the TranSignal TF Protein Array when screening protein - DNA interactions





Typical results obtained with the TranSignal TF Protein Array IV. The TF protein array IV was incubated with the biotinylated PCR product of the rat PEPCK Promoter Region (-1 to -465) alone (Figure A) and both biotinylated and unlabeled PEPCK $promoter\ (Figure\ B.).\ Images\ were\ acquired\ using\ Fluor\ Chem^{\texttt{TM}}\ imager\ (Alpha\ Innotech).$ Specific Protein/DNA interactions (boxes) were competed out with unlabeled probe, whereas non-specific binding of the probe was not.

20 TranSignalTM TF Protein Array

NOTES: